
Alterations on Rodent Ultrasonic Vocalizations and Consequent Effects on Mate Selection

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Abstract

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In this study, the effects of altering different components of a rodent's mating calls and their consequent effects on mate selection were explored. Rodents have become an established animal model in laboratory studies due to their inexpensive cost and common mammalian attributes shared with humans. While amphibian studies examined female selection of male mates based on acoustic features, to this date no mammalian model has been used to investigate these questions. From previous research, we know that unilateral dopamine depletion in the nigro-striatal pathway within the brain results in a drop in intensity and bandwidth of its calls. This is seen commonly in patients that suffer from Parkinson's Disease: soft voice with monotonic pitch. My project seeks to go one step farther and ask whether it is the intensity, bandwidth, or both which modulates a female's preferential behavior towards a mating call. To answer this question, our lab constructed an experiment involving two new sets of stimuli (only intensity reduced: -4dB and only bandwidth reduced) to be tested on two sets of rats: sexually-experienced and sexually-naïve. Using a T-maze setup to track our female rats' preferential behaviors, we observed that both sexually experienced and inexperienced female rodents show a high preference for the normal intensity calls over those of low intensity. However, when given a choice between normal calls and low-bandwidth altered calls, the sexually experienced rats preferred the normal calls whereas the sexually naïve rats seemed to exhibit no such preference. These results help us to conclude that sexually experienced female rats prefer normal, or relatively higher, intensities and bandwidths of calls. However, the results from testing these

calls on sexually naïve female rats broaches a new question of the role of sexual experience in determining preference in mating calls. Further research can be conducted investigating whether the preference would differ had the females undergone sexual experience with Parkinson's diseased rats who produce low intensity and low bandwidth calls which could elucidate the role of sexual experience in discerning mating calls.

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Introduction

Only recently was it discovered that laboratory rodents call to each other ultrasonically in a 22-80kHz range, falling outside of normal human hearing range of 20Hz-20kHz. Consequently, this ultrasonic vocalization or “USV” has become an important behavioral tool in analyzing rat models of depression and anxiety (Mallo et al., 2007), fear conditioning (Swiergiel et al., 2007), mother-pup interactions (Branchi et al., 2001), reward systems (Burgdorf et al., 2007), and recently as a model for sensorimotor deficits associated with Parkinson’s disease (Ciucci et al., 2007).

In general, rats produce two types of calls which are categorized by frequency and complexity of the waveform—one being at 50kHz the other at 22kHz. The 22kHz call is produced during an aversive state such as being defeated by a cage mate, while the 50kHz call is made during a positive responsive state, such as during social interaction, and is generally more complex. Additionally, 50kHz vocalization is correlated with sexual motivation and is produced more frequently by males as they increase sexual experience with receptive (estrous) females.

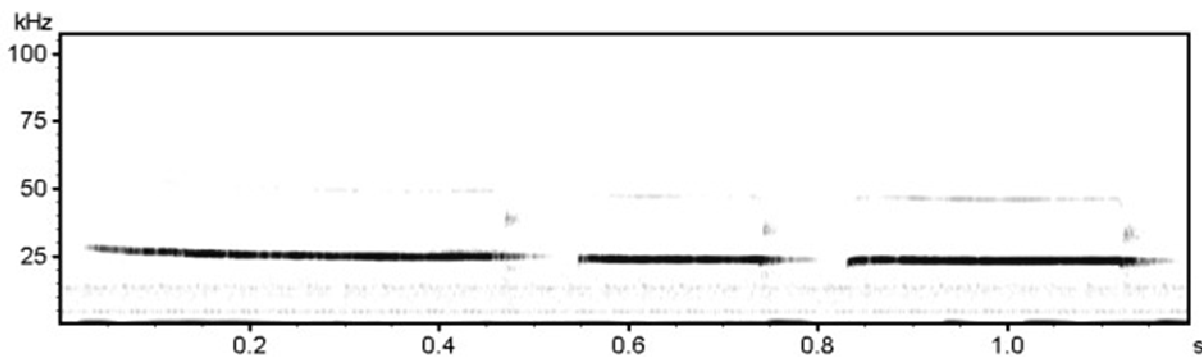


Fig. 1: Example of a simple (20kHz) rat call. Note that these calls are long (lasting up to seconds) and narrow-banded.

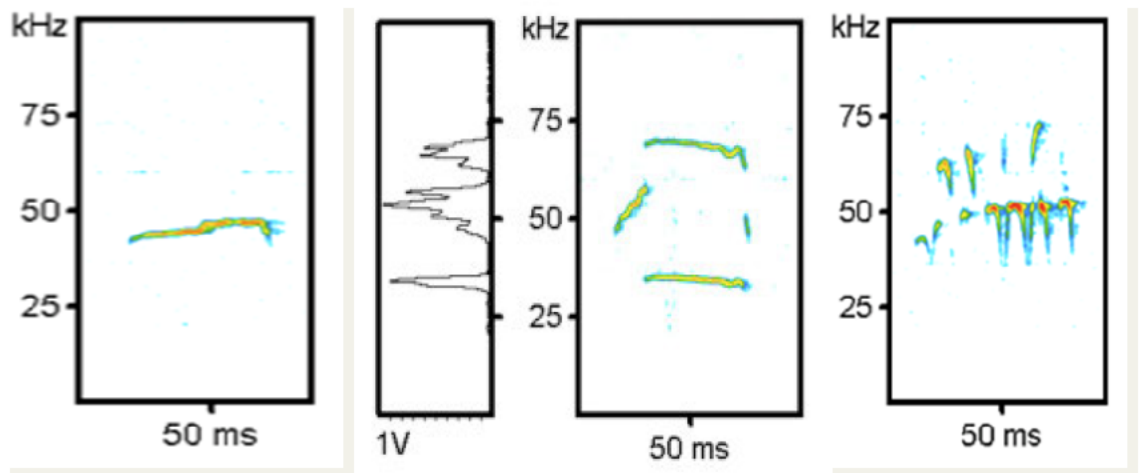


Fig. 2: Examples of 50kHz complex calls. While there are seven categories of complex calls, the majority of calls recorded from our experiments recorded to these three categories: flat, harmonic, and frequency modulated (from left to right).

A landmark study conducted in 2007 demonstrated that killing dopamine neurons in pre-substantia nigra to model neurodegeneration in PD led to a degradation of a rat's USV acoustic signal, especially those of the 50kHz calls (Ciucci et al., 2007). In this study, Ciucci et al. compared USV calls of normal rats to those of the PD model rats. They found that dopamine depletion in one hemisphere of a rat's brain (caused by infusion of 6-OHDA, a dopamine toxin) causes adverse effects on certain components of the call—specifically a decrease in that rat's mating call bandwidth and intensity. The quality of ultrasonic vocalization was degraded without a reduction

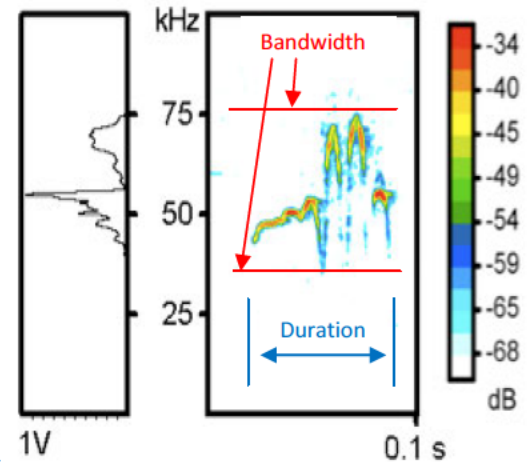


Fig. 3: Sample breakdown of an ultrasonic vocalization. Components include duration, bandwidth, and intensity (described by the color).

in the number of calls it produces. This notion of call quality analysis sets the foundation for our experiment to explore the effects of altering different components of an ultrasonic vocalization individually.

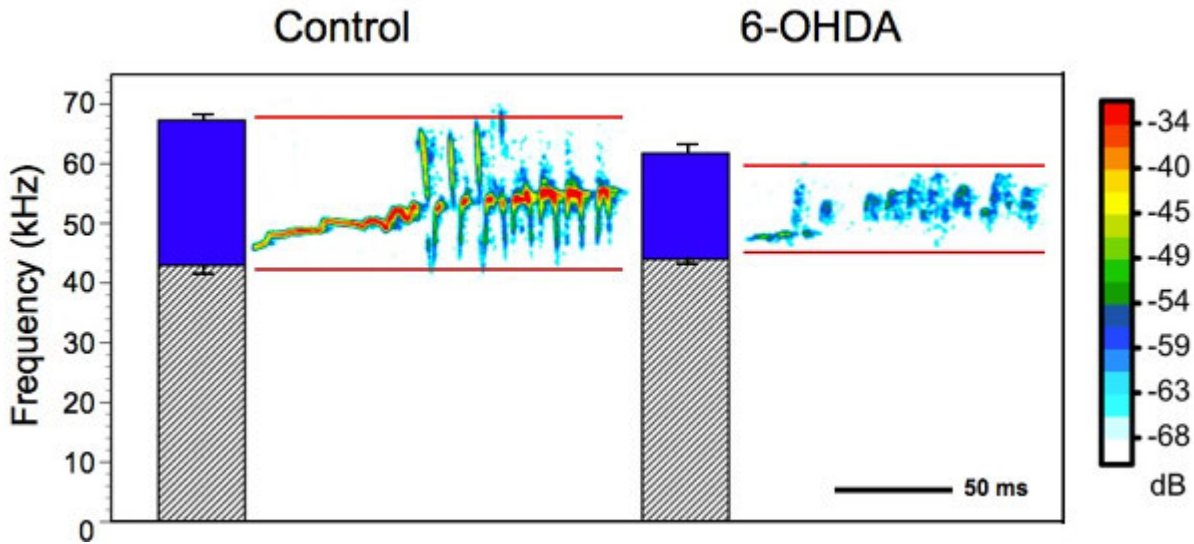


Fig. 4: Representative sonograms of the trill-type call for control and unilateral 6-OHDA infusion. Average and standard errors of the maximum and minimum peak frequency are represented by the bar graphs. Solid color between the maximum and minimum peak frequency indicate the bandwidth of the call. Also note the decrease in intensity (dB) levels. *Ciucci, Ma, Fox, et al. (2008) Parkinson and Related Disorders.*

Regarding the behavior portion of our research, previous studies have demonstrated that preference mate choice tests are successful in indicating the attractiveness of a certain trait. Female Tungara Frogs for example, are more attracted to complex mating calls from a male frog as compared to a simple call; when exposed to both calls, the female frogs spent more time on the complex call side versus the simple call side of an enclosure (Ryan 1980). Even in rodents, different male calls have often elicited different behavioral responses in the females (Pomerantz et al., 1983; White et al., 1993). Furthermore, studies have shown that sexually experienced male rats consistently produce higher numbers of ultrasonic vocalizations in the presence of a female in estrous. Utilizing these findings, our laboratory conducted preliminary experiments which tested the effects of altered mating calls on female behavior. To do so, our laboratory presented mating calls of the dopamine depleted rats versus normal rats to female rats in estrous. Our results from this preliminary experiment indicated that overwhelmingly, both sexually

experienced and naïve females prefer the normal rat's mate call, which was characterized by a higher bandwidth and intensity over the dopamine depleted or PD (Parkinson's Disease) call. These findings ultimately lead us to investigate the alterable components of the mating call and the consequent effects of these adjustments on both sexually experienced and sexually naive female rat behavior.

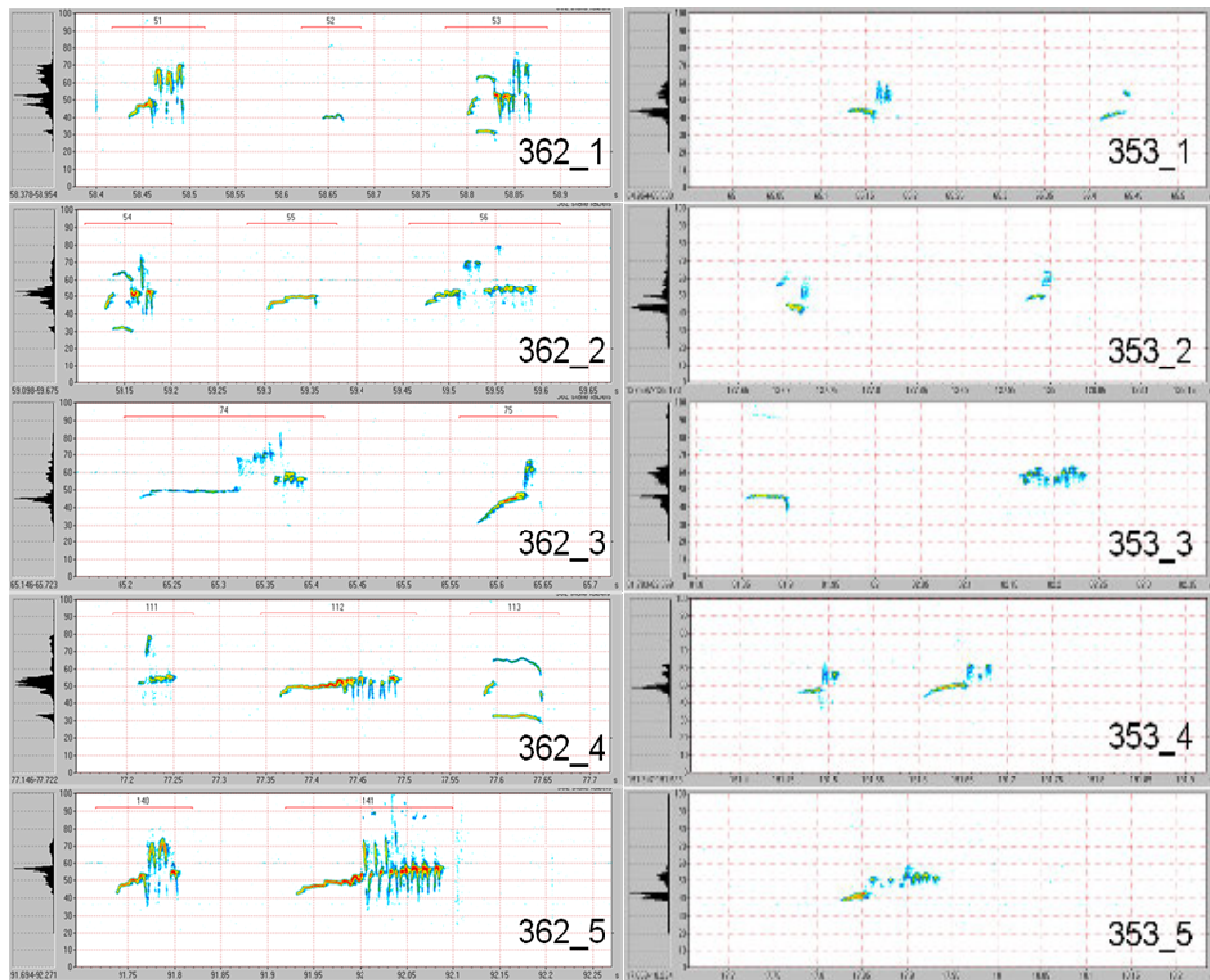


Fig. 5: Left: Five sample mating calls recorded from normal males recorded in presence of an estrous female; Right:

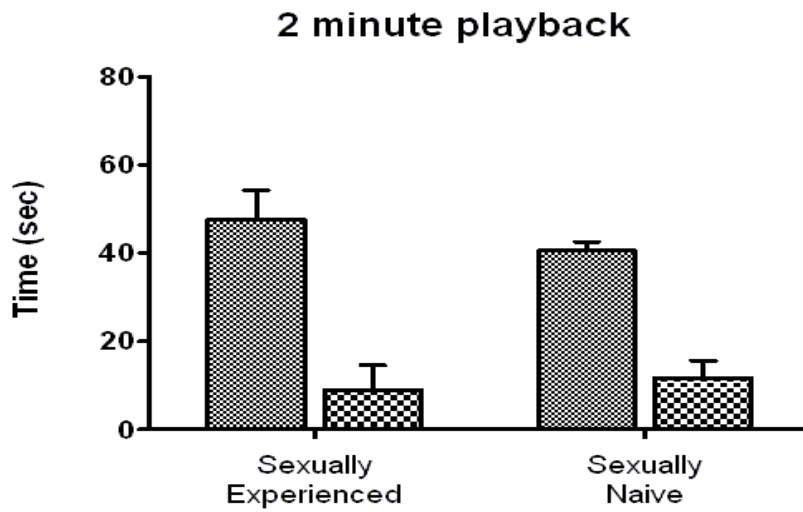


Fig. 6: Shown are the average times spent investigating each call for each rat. Note that both sexually experienced and naive rats show a relatively similar strong preference for the Normal calls (left bar) as compared to the PD or dopamine depleted calls (right bar). *Ma, et al. (2009) (submitted)*.

Materials and Methods

Animals and housing

26 female Long-Evans rats, obtained from Charles River and aged 4 months were used for playback experiments. All animals were pair-housed in groups of two in polycarbonate cages on a reversed 12:12 hour light: dark cycle. All testing occurred during the dark period of the cycle. Food and water were available ad libitum. All experiments conducted were approved by the University of Texas Animal Care and Use Committee.

Sexual Experience

Female rats were randomly selected into sexually experienced (n=16) and sexually naive (n=10) groups. Females were brought into estrous through i.p. injections of 10 µg of estradiol (Sigma, USA) and 500 µg of progesterone (Sigma, USA) at 48 hrs and 4 hrs prior to behavioral testing, respectively. The sexually experienced group was given five opportunities over 15 days during estrus to gain sexual experience with sexually experienced males. Both groups were habituated to a polycarbonate T-maze in 10-min sessions 5 times prior to the experiment.

Acoustic Stimuli

Mating calls of control and unilateral DA-depleted male rats for playback were selected from recordings from our previous study. A 20 second clip of USV mating calls was excerpted from randomly selected, but representative, control male recordings. These recordings were then adjusted to accurately mimic naturally reduced intensity or bandwidth calls, looped, and presented continuously up to 3 minutes through a high speed D/A board (PCI-6221, National Instrument, Austin, TX) connected with an ultrasonic speaker (Fountek, China) (Figure 7).

Speakers broadcasting USVs stimuli from control vs. DA depleted rats were randomized between sessions. Intensity was calibrated to match our previous mating call recording study for both stimuli. Only 50-kHz range calls were present in the sample. Sonograms from both stimuli are shown in Figure 5.

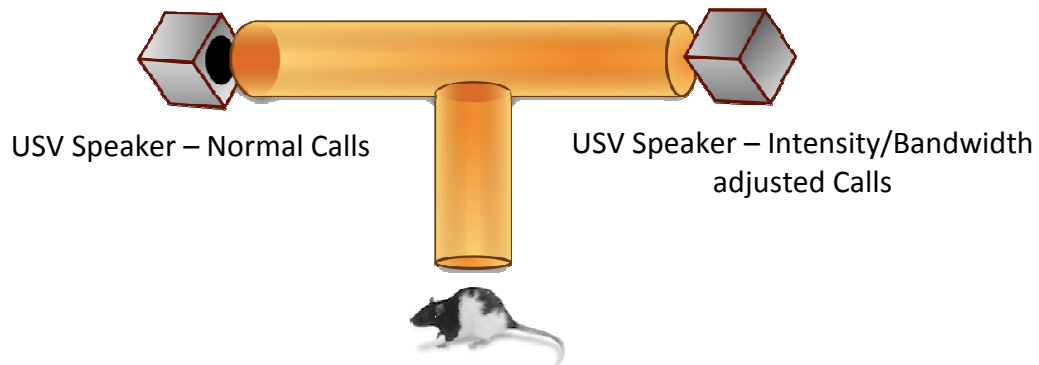


Fig. 7: Two-speaker USV playback setup.

Behavioral testing

Behavioral testing was carried out in low red lighting for all animals. A 5-min period of silence in the T-maze preceded each 1 and 3-min playback phase for each female (speaker arms: 40 cm; neutral arm: 36 cm). Behavior was recorded from an infrared camera (Panasonic, Japan). Time spent exploring the left and right sections of the box was assessed during the 1 and 3-min tests. Data indicated marked female preference for normal male calls. The T-maze was cleaned with 70% alcohol before each test.

Results

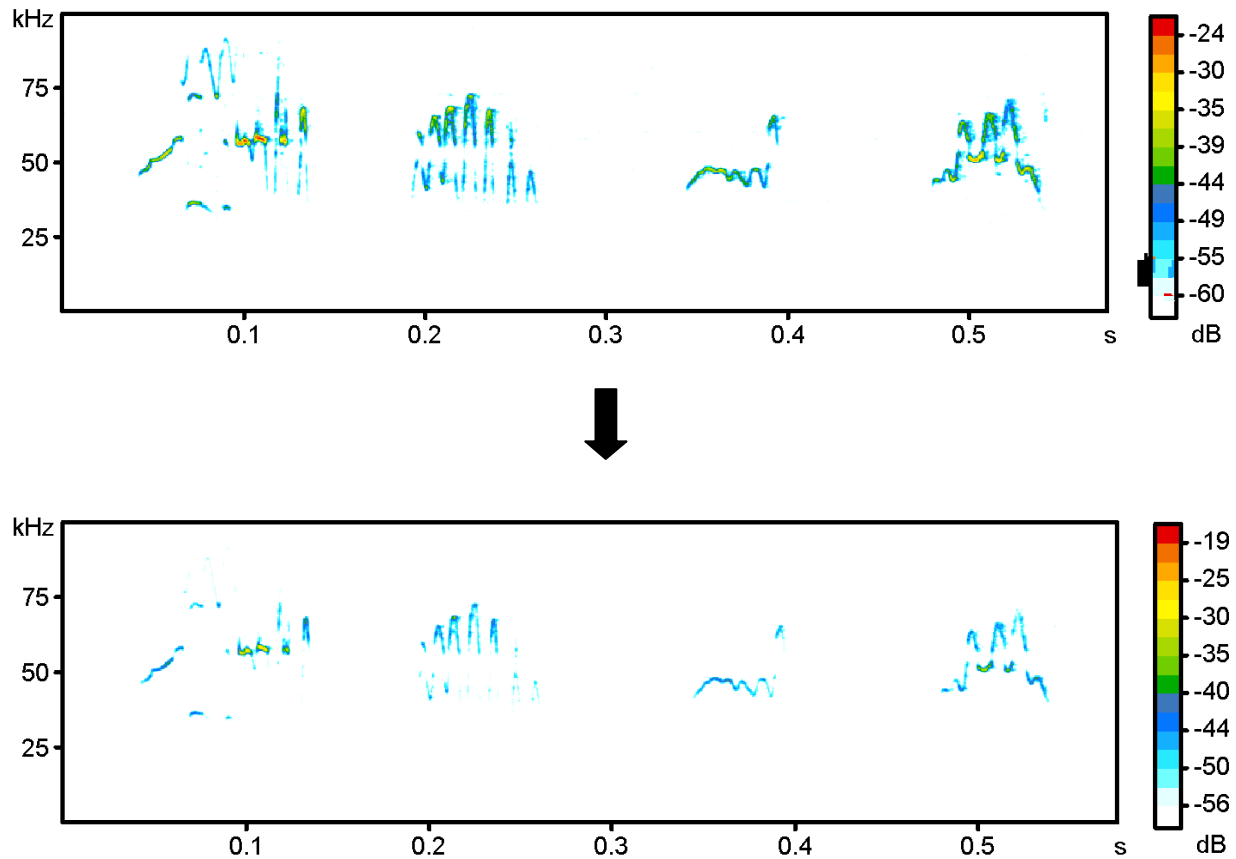


Fig. 8: Sample of an ultrasound from control to intensity-reduced. Control calls are artificially altered to mimic PD calls while maintaining constancy throughout the experiments. Note that the bandwidth is kept constant.

When we subjected the sexually naïve and experienced rats to the intensity reduced playback using the T-maze construction over the span of two minutes as described in “Materials and Methods”, we noticed that despite sexual experience or inexperience, both types of female rats preferred the control call to that of the altered call with a reduced intensity with a relatively similar statistics: sexually experienced rats averaging 17.2 seconds (investigating the control) over 3.8 seconds (spent investigating the intensity reduced) while the sexually naïve rats averaged 11.3 seconds (control) versus 4.0 seconds (intensity reduced). The ratios of time spent

investing each call (experience/naïve) are relatively close between the experienced and naïve: 4.53 for the sexually experienced compared to 2.83 for the sexually naïve.

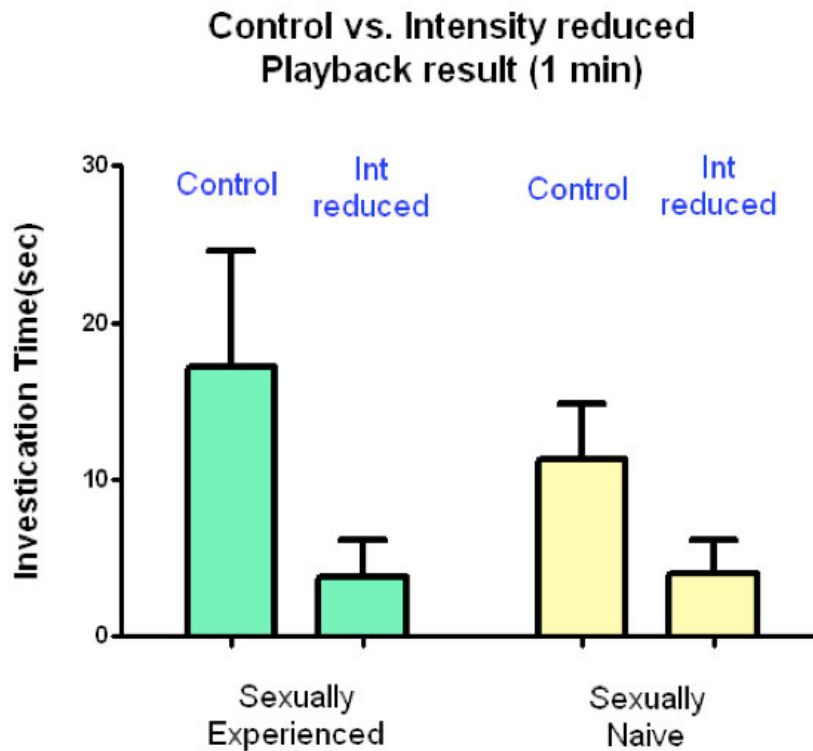


Fig. 9: Graph depicting the relative behavioral responses of sexually experienced and naïve females to a control or intensity reduced stimulus.

However, when we tested the preference for the control call versus the bandwidth reduced call, the sexually experienced and sexually naïve rats responded very differently. In the sexually experienced rats, we saw that they spent on average 11.7 seconds investigating the normal call side, whereas in contrast they spent on average only 0.3 seconds investigating the bandwidth reduced call. The sexually naïve rats, however, did not behaviorally respond with as great of a contrast spending 13.0 seconds on the control side and 9.1 seconds on the bandwidth reduced side (Figure 11). The ratios (time investigating control/bandwidth reduced) consequently show a much greater discrepancy: 35.33 (experienced) compared to 1.43 (sexually naïve).

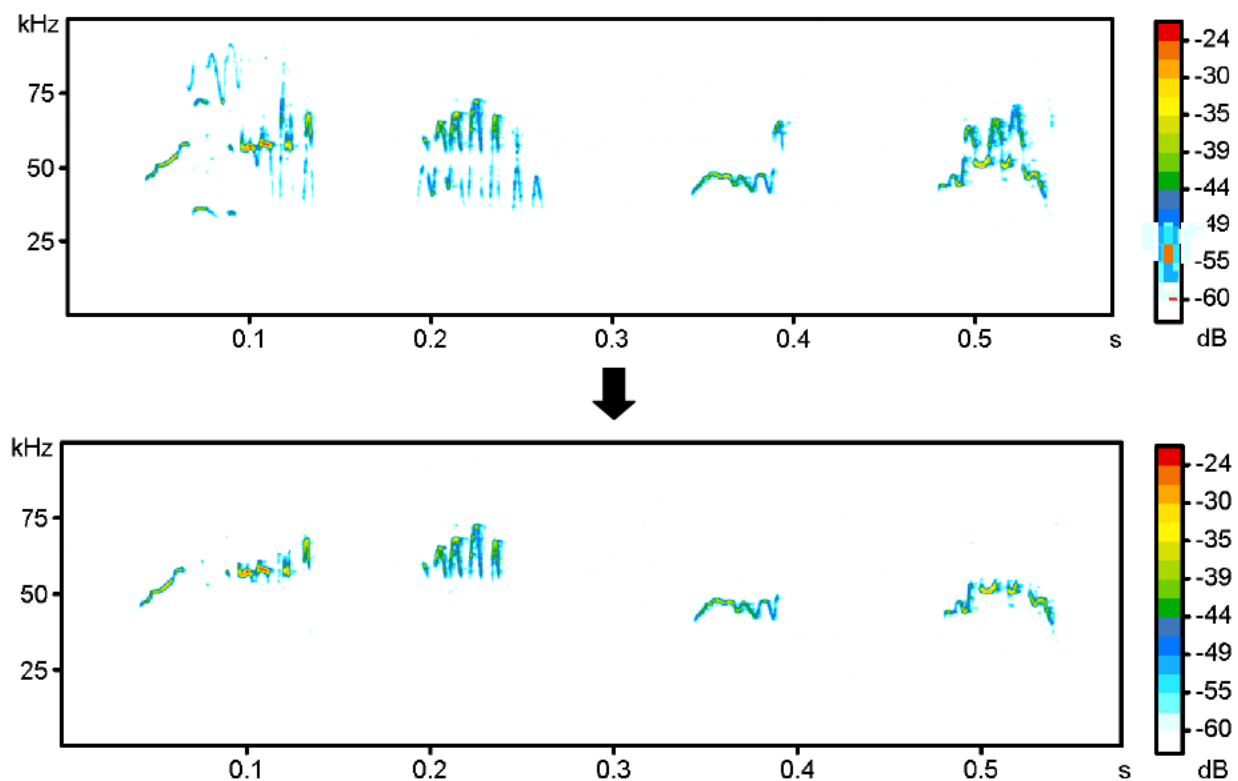


Fig. 10: Sample of an ultrasound from control to bandwidth-reduced. Control calls are artificially altered to mimic PD calls while maintaining constancy throughout the experiments. Note that the intensity levels are kept constant.

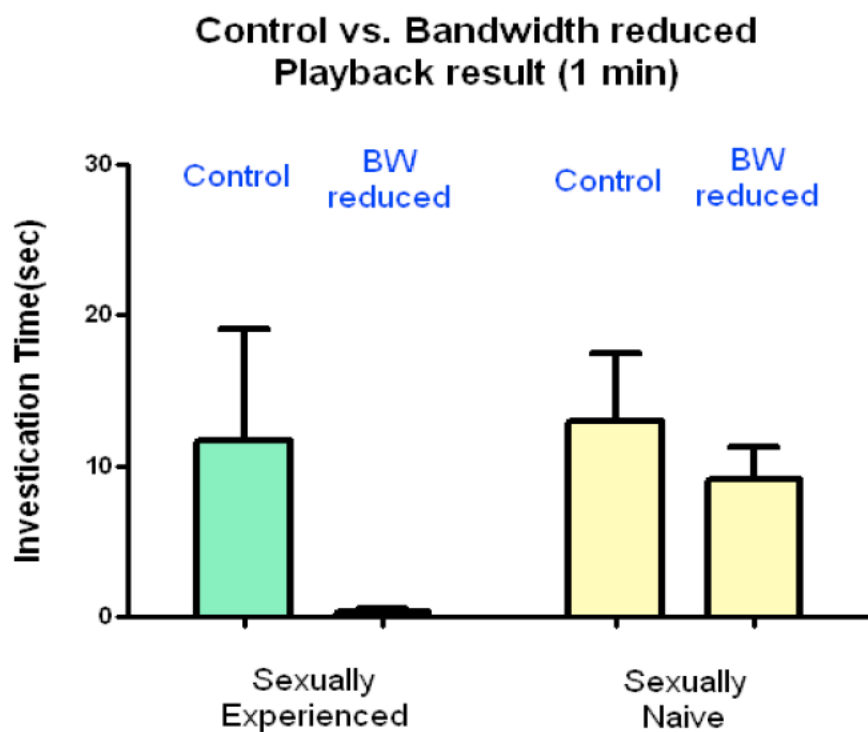


Fig. 11: Graph depicting the clear bias for the control call over a bandwidth reduced call in the sexually experienced rats. This is in contrast to the relatively even spread of investigation time between the two calls in sexually naïve rats.

Intensity Playback 3 minute Session

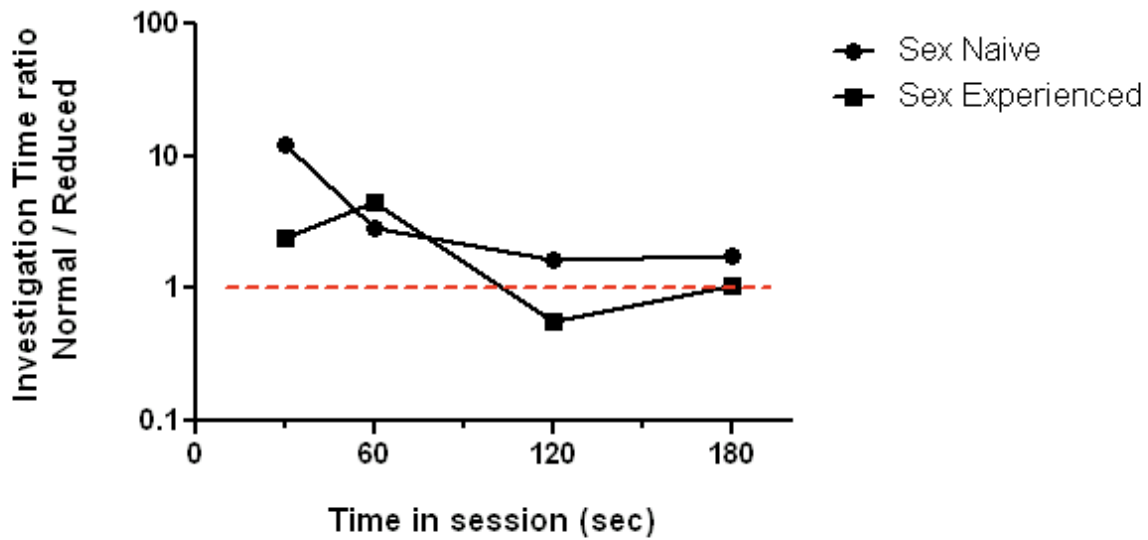


Fig. 12: Shown is a graph depicting the ratio of time spent investigating the normal call versus the intensity reduced call over a three minute playback session. Note that both the sexually naive and experienced rats follow similar trends in preferring the normal calls over the intensity reduced calls. The dotted red line represents a one-to-one ratio of time spent investigating each stimulus.

Bandwidth Playback 3 minute Session

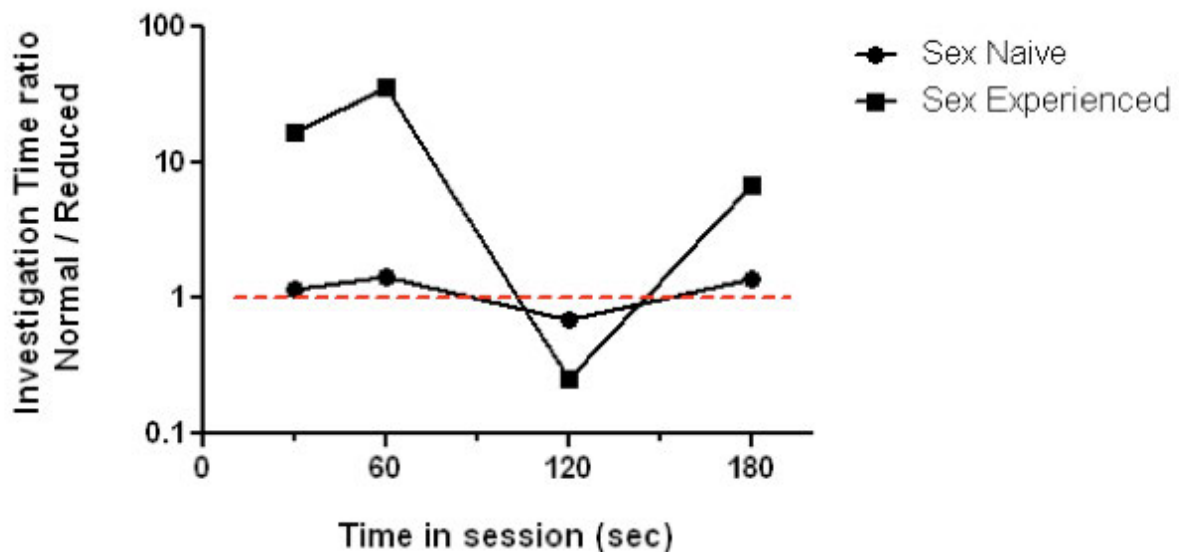


Fig. 13: Shown is a graph depicting the ratio of time spent investigating the normal call versus the bandwidth reduced call over a three minute playback session. See the discrepancy between the sexually experienced rats that they overwhelmingly prefer the normal call over the bandwidth reduced, whereas the naïve females hover with a relatively even ratio. The dotted red line represents a one-to-one ratio of time spent investigating each stimulus.

Depicted in Figures 12 and 13 are results from our 3-minute playback sessions with the same calls looping, but the focus of this experiment was to track and contrast the overall trends of preference for the sexually experienced and naïve rats. As shown in Figure 12, when given a choice between normal and intensity reduced calls, female rats tend to prefer the normal calls. It is possible that this lower intensity denotes a perhaps a more distant call or a lower-quality mate. In Figure 13 however, we see that the ratios of time spent investigating the normal calls to the bandwidth reduced calls are largely contrasted. The naïve rats spend nearly an equal amount of time investigating both the normal and bandwidth reduced whereas the sexually experienced rats favor the normal call by a much great ratio.

Discussion & Conclusions

A foundation for quality analysis of ultrasonic vocalization (USV) in laboratory rodents was developed when USV calls of dopamine depleted rats (Parkinsonian rats) were compared with normal rats' calls (Ciucci et al., 2007). Noting a discrepancy existed between these two types of calls, Ma et al. utilized a playback paradigm to further investigate the effects of these degraded calls on mate preference in female rats (Ma et al., 2009). With the use of mating calls, as they are commonly employed by other species in sexual selection (Vignal et al., 2006; Bass et al., 1997), Ma et al. tested females' mating preference based solely on their responses to a male's call quality—normal call vs. 6-OHDA or “PD-like” call. Ma et al. noted that female rats responded differently to these two types of call. This result further inspired my curiosity on which component of mating calls, either bandwidth or intensity, influenced the decision making in the female rat's brain that was further displayed in her behavior. Our study yielded interesting results, especially in regard to the significant effect altering bandwidth had on preference. It was predicted that decreasing intensity would yield a lower preference, perhaps because a decrease in intensity translates to a softer and seemingly more distant call, which is biologically more risky during mate searching with potential predators close by. However, whether the degradation of bandwidth would have an effect on the female rats was unsure. As shown in the Results section, the sexually experienced rats showed a large preference for the normal call as compared to the nearly equal preference given to both calls by the sexually naïve rat. This finding was of particular interest because it sets up further questions regarding this apparent bias, with sexual experience being the only variable influencing behavior outcome.

Future areas of study could ask whether this preference is experience induced or simply innate but in need of a trigger. In this experiment, the females in question would be mated with

PD rats which produced low bandwidth calls, and then subjected to the T-maze test to see whether the behavior would change. Taking this concept one step farther, one could use biological analysis to clarify whether this apparent lack of preference seen in naïve female rat behavior is also consistent in the brain. Using gene markers, we could track the expression of specific genes namely IEGs, or immediate early gene, whose proteins are released in the brain, when we subject the females to certain external stimuli—in this case a normal call or a low bandwidth call (Sadananda et al., 2008). From this brain analysis, our lab can expect one of two outcomes, either of which helps to identify the role of experience in sexual preference for rats. If the brain slices of sexually naïve rats reveal that brain activity remains constant upon exposure to normal calls and to low-bandwidth calls, our lab has strong evidence to attribute sexual preference in rats to previous sexual experience—as the experience is the only variable to explain the bandwidth preference results. However, if the brain slices reveal a stronger biological response to the normal call over the low-bandwidth call, even in the sexually naïve rats, we have evidence that there exists some sort of innate preference for certain types of mating calls over others—even if it's not behaviorally triggered or interpreted yet. With either outcome, we will have opened doors to important further studies regarding sexual preference and the links between biology and behavior especially in mammalian systems. And while we acknowledge that our study has set a good foundation, further questions must be asked to clarify our results so that our research and scientific knowledge on this subject can continue to move forward.

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